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Study on the Occurrence of Bovine Mastitis in Addis Ababa Dairy Farms and Associated Risk Factors

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Abstract: A cross-sectional study was conducted in large and small scaled dairy farms in Addis Ababa from October, 2011 to March, 2012. The objectives were to assess the prevalence of clinical and subclinical mastitis and isolate bacterial pathogens in lactating cows. A total of 300 lactating animals comprising 216 Holistine Friesian, 13 Jersey and 71 cross breed cows were randomly selected from the sampling unit and screened using CMT test for the evidence of subclinical mastitis. The overall prevalence of subclinical and clinical mastitis was 43.3 and 22% at cow level and 26.8 and 15.1% at quarter level respectively. California Mastitis Test (CMT) positive milk sample were subjected to bacteriological examination. Out of 146 positive samples, 109 were cultured positive. Among this cultured positive samples, five genera of bacteria and mixed bacterial infection were isolated. The most prevalent mastitis causing agents isolated in this study were (31.19%) *Staphylococcus aureus*, (26.6%) coagulase negative staphylococci, (11.93%) *E. coli*, (8.26%) *Streptococcus*, (5.5%) *Klebsiella* and (2.75%) *Corynebacterium* and mixed infections; (1.83%) *Staphylococcus* and *Streptococcus*, (4.59%) *Staphylococcus* and *Corynebacterium*, (4.59%) *Staphylococcus* and *E. coli* and (2.75%) *Corynebacterium* and *E. coli* were identified. Risk factor; parity (P^2 *= 27.955; df =1 and P. value = 0.000)*, lactation stage (P^2 *= 21.136; df=1 and P. value = 0.007*) showed statistically significant association with the occurrence of bovine mastitis in lactating cows. However, there was no statistically significant association observed between breeds. In conclusion it could be better if dairy farmers and enterprises use the appropriate method of prevention and control methods to reduce the prevalence of mastitis and there by increasing production of milk and its quality.

Key words: Addis Ababa % Bovine % Mastitis % Parity % Pathogen And Prevalence

INTRODUCTION

Ethiopia has the largest livestock production in Africa with an estimated 35 million tropical livestock units (TLU) including 51 million cattle, 42 million sheep and goats and 7 million equines [1, 2]. In contrast to the huge livestock resource, the livestock productivity is however, found to be very low. The major biological and socio-economical factors attributing to the low productivity include: the low genetic potential and performance, poor nutrition (in quality and quantity terms), the prevailing of different diseases, traditional way of husbandry systems and inadequate skilled manpower, among others [3].

Dairy production is a biologically efficient system that converts feed and roughages to milk. Milk is a very nutritional food that is rich in carbohydrate, protein, fat, vitamins and minerals [4]. There are several types of diseases which affect the wellbeing of livestock population; among which mastitis is the common and costly disease and as well it is considered as the most complex disease because of its multifactorial causation [5]. Mastitis is the most costly infectious disease of dairy cattle. The prevalence of mastitis in dairy cattle is relatively high. Subclinical mastitis is the main form of mastitis in modern dairy herds, exceeding 20 to 50% of cows in a given herd [6].

Mastitis is inflammation of parenchyma of mammary gland characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissue [7]. Bovine mastitis is the single most common cause for antibacterial use in lactating dairy cattle [8]. Lumpy skin disease, food and mouth disease and ephemeral fever are the most important viral causes of mastitis [9]. Many microbial species that are common

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causes of bovine mastitis, such as *Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae and Staphylococcus aureus* also occur as commensal or pathogens of humans [10].

To simplify understanding of the mastitis complex, it is useful to consider that three major factors are involved in this disease: the microorganisms as the causative agent, the cow as host and the environment, which can influence both the cow and the microorganisms [11]. In the study area there is a high level of milk and milk products consumption but successful and satisfying work has not yet developed. Therefore, the objectives of this paper were to determine prevalence of bovine mastitis in Addis Ababa in selected dairy farms and determine major etiologies for bovine mastitis and associated risk factors.

MATERIALS AND METHODS

Study Animals: A total of 216 Holiestin Frisian (HF), 13 Jersey and 71 cross lactating cows in selected dairy farms in Addis Ababa from October 2011 to March 2012 in a total of 28 dairy farms were used as sampling unit in determining the prevalence of mastitis. These farms have a minimum of 3 and a maximum of 40 lactating cows. The cows are managed under intensive and semi intensive management system.

Study Design: A cross-sectional type of survey was conducted on 300 lactating cows of different breed in large and small scaled dairy farms in Addis Ababa. Those animals were examined directly at quarter level for clinical manifestation and indirect test (CMT) for subclinical prevalence. In this study animal prevalence of clinical and sub-clinical mastitis was determined using clinical observation, CMT result and microbiological examination from strong positive CMT result samples.

Sample Size and Sampling Method: Simple random sampling method was used for sampling representative study animals. The sample size was determined according to Thrusfield [13] at 95% confidence interval (CI) and 5% precision with the expected prevalence of 24.3% by assuming that there was no previous study in the area. Simple random sampling method was considered to select the individual dairy cow. Therefore, by substituting the values in the above formula, n =283 was the sample size. However, to increase the truth values the sample size was increased to 300.

Milk sample collection, handling and transportation: Aseptic procedures were used for collecting milk samples. The time chosen for milk sample collection was before milking. Data collection included type of dairy husbandry system, breed, parity and lactation stage [1]. Udder and especially teats were cleaned and dried before sample collection. Each end was scrubbed vigorously with a pledge of cotton moistened with 70% ethyl alcohol. A separate pledged of cotton was used for each teat. The first few streams of milk were discarded and 10ml of milk was collected in to horizontally hold vial. After collection, the sample was placed in an icebox and transported to the Veterinary Microbiology Laboratory for analysis [10].

California Mastitis Test (CMT): To detect the prevalence of mastitis milk samples were collected aseptically from each quarter and brought to the laboratory with tightly closed plastic vials and examined physically for clinical mastitis and tested using California Mastitis Test (CMT) for subclinical mastitis [9].

Bacteriological Isolation: Milk samples were bacteriologically examined according to the procedures employed by Quinn *et al*. [14]. In refrigerated milk samples, bacteria might be concentrated in the cream layer and held with in clumps through palpation to detect possible fibrosis or cardinal of fat globules [15]. Hence dispersion of fat and bacteria was accomplished by warming the samples at 25°C for 15 minutes and shaking before plating onto a standard bacteriological media. A loopful of cows' milk with clinical mastitis was taken to check sample collected from each infected quarter involved. Information related to the previous health was inoculated separately on to MacConkey agar and blood agar base enriched with 7% defibrinated bovine blood. The inoculated plates were then incubated aerobically at 37° C for 24 to 48 hours. Identification of bacteria on primary culture was made according to Quinn *et al*. [14].

Data Collection: Data on each cow was collected in a format designed for this purpose. Risk factors considered were breed, parity, stage of lactation, any lesion, husbandry system and other abnormalities (blindness, swelling and any change in the milk). Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub clinical mastitis.

Data Analysis: Data collected from each study animal and laboratory works were coded in to appropriate variable and enter in Microsoft Excel spread sheet. Then statistical analyses were performed using SPSS statistical software package version 17.0. Effects of specific variables

(breed, parity, stage of lactation and udder/teat injuries) were investigated on prevalence of mastitis by using chi-square (P^2) test.

RESULTS

Prevalence: Both clinical and sub-clinical mastitis prevalence was determined cross-sectionally at cow level and quarter level based on clinical examination and California Mastitis Test (CMT). Out of a total of 1200 quarters examined from 300 lactating dairy cows, 50 (4.2%) were blind/ nonfunctional quarters. Whereas 503 (41.9%) were positive for mastitis through clinical examination and CMT test results.

From 300 lactating cows examined, 66 (22%) had got clinical mastitis characterized by signs of inflammation, change in the viscosity and color of milk and blockage of teats. Among these 66 clinical cases, 35 had got blind teats and 31 were active case of mastitis respectively. Out of 300 lactating cows screened using (CMT) to detect the presence of mastitis, 130 (43.3%) were found positive for subclinical mastitis.

The overall result showed that the prevalence of clinical and sub-clinical mastitis was 22 and 43.3% at cow level and 15.1 and 26.8% at quarter level respectively.

Risk Factors Affecting Prevalence of Mastitis: Association of different potential risk factors (Breed, parity, lactation stage) with mastitis prevalence was checked by chi-square test. The results of association of risk factors with occurrence of mastitis were indicated in table 1.

The overall prevalence of mastitis at the level of quarter was 41.9%, among this, the prevalence of subclinical and clinical mastitis was 26.9 and 15.1% respectively. Quarter location was statistically significant with mastitis prevalence (P^2 *= 308.921; P. value = 0.000*). The prevalence of subclinical and clinical mastitis was higher in front quarter than the hind quarters, which was indicated in (Table 2).

The most prevalent mastitis causing agents isolated in this study from 109 positive cultures were (31.19%) *Staphylococcus aureus*, (26.6%) coagulase negative staphylococci, (8.26%) *Streptococcus*, (11.93%) *E. coli*, (2.75) *Corynebacterium* and (5.5%) *Klebsiella.* Mixed infections were (1.83%) *Staphylococcus* and *Streptococcus*, (4.59%) *Staphylococcus* and *Corynebacterium*, (4.59) *Staphylococcus* and *E. coli* and (2.75%) *Corynebacterium and E. coli.*

Table 1: Occurrence of mastitis and associated risk factors

Risk factors	Mastitis					
Breed	Subclinical	Clinical	Total	Total prevalence	P^2	P.value
Holstein	97(32.33%)	48(16.0%)	145	48.3%(145/300)	3.439	0.455
Jersey	5(1.67%)	1(0.03%)	6	2% (6/300)		
Cross	28(9.33%)	17(5.67%)	45	15% (45/300)		
Parity						
One calf	28(9.33%)	11(3.67%)	39	13% (39/300)	27.955	0.000
Two calves	33(11%)	14(4.67%)	47	15.67% (47/300)		
Three Calves	35(11.67%)	16(5.33%)	51	17% (51/300)		
Four calves and above	34(11.33%)	25(8.33%)	59	19.67%(59/300)		
Lactation Stage						
Below One month	19(6.33%)	5(1.67%)	24	8% (24/300)	21.136	0.007
One to three months	45(15%)	19(6.33%)	64	21.3% (64/300)		
Four to six months	24(8%)	27(9%)	51	17% (51/300)		
Seven to nine months	28(9.33%)	8(2.67%)	36	12% (36/300)		
Ten months and above	14(4.67%)	7(2.33%)	21	7% (21/300)		

NB. The result indicates the prevalence of mastitis with respect to parity was found to be statistically significant (P^2 *= 27.955; df =1 and P. value = 0.000*). The risk of mastitis was increased with increasing parity number. This was indicated in (table1). Prevalence of mastitis with respect to stage of lactation was statistically significant (P^2 *= 21.136; df =1and P. value = 0.007*). Parity and lactation stage were statistically significant with mastitis prevalence. However, prevalence of mastitis was insignificant with P > 0.05 in breed.

Table 2: The occurrence of mastitis in the examined different quarters

Quarters	Form of mastitis		Total no of animals	Total no of quarters	Prevalence at quarter level
	Subclinical	Clinical			
Front Right	9	1	10	10	0.83%
Front left	4	4	8	8	0.67%
Hind Right	7	2	9	9	0.75
Hind Left	4	3	7	7	0.58%
All quarters	27	23	50	200	16.67%
FL, FR and HR	11	4	15	45	3.75%
FR, FL and HL	9	4	13	39	3.25%
FR, HR and HL	8	4	12	36	3%
FL, HR and HL	6	3	9	27	2.25%
FR and FL	10	3	13	26	2.17%
FR and HR	6	2	8	16	1.33%
FR and HL	4	4	8	16	1.33%
FL and HR	7	3	10	20	1.67%
FL and HL	6	1	7	14	1.17%
HR and HL	11	4	15	30	2.5%
Total	130	66	196	503	41.9%

P^2 = 308.921; P. value = 0.000

Note: FL= Front Left, FR= Front Right, HL= Hind Left and HR= Hind Right.

DISCUSSION

The epidemiological studies in this investigation were applied through combination of the CMT with bacteriological cultures, because subclinical mastitis was defined as when mammary glands without clinical abnormalities giving apparently normal milk but was bacteriologically positive and with positive CMT [16].

In the present study the overall prevalence of clinical and subclinical mastitis, out of 300 lactating cows examined was 22 and 43.3% at cow base and 15.1 and 26.9% at quarter level respectively. In this study the prevalence of subclinical mastitis was higher than that of clinical mastitis. This could be due to the reason that in Ethiopia subclinical mastitis receives little attention and efforts have been concentrated only on the treatment of clinical cases [17]. Of the 1200 quarters examined, 50 were blind, which may be an indication of a serious mastitis problem on the respective farms and of the absence of a culling programmer that can serve as a means to remove the source of this mammary pathogens for other cows.

Bacterial Isolates: Out of 146 CMT and clinically positive samples, 109 were cultured positive. Among this cultured positive samples, five genera of bacteria and mixed bacterial infection were isolated (Table 3).

Table 3: Bacterial isolates identified from mastitis positive samples

Etiological agents	Form of mastitis		Prevalence (%)	P^2	P value
	Subclinical	Clinical			
Staphylococcus aureus.	25(8.33%)	9(3.0%)	11.33%(34/300)	99.018	0.000
Other *Staphylococcus* Species	20(6.67%)	9(3.0%)	9.67%(29/300)		
Streptococcus	5(1.67%)	4(1.33%)	3.0%(9/300)		
E. Coli	5(1.67%)	8(2.67%)	4.33%(13/300)		
Corynebacterium	1(0.33%)	2(0.67%)	1.0%(3/300)		
Klebsiella	4(1.33%)	2(0.67%)	2.0%(6/300)		
Staphylococcus and Streptococcus	2(0.67%)	0(0.0%)	0.67%(2/300)		
Staphylococcus and Corynebacterium	4(1.33%)	1(0.33%)	1.67% (5/300)		
Staphylococcus and E. coli	4(1.33%)	1(0.33%)	1.67% (5/300)		
Corynebacterium and E. coli	2(0.67%)	1(0.33%)	1.0%(3/300)		

A number of prevalence studies of bovine mastitis were carried out in Ethiopia. Out of them 19 and 5.3% in Central Ethiopia [18], 5.3 and 1.9%[17], 34.3 and 5.3% in Addis Ababa [19], 30.2 and 5.5% in urban and Peri urban dairy production system in and around Addis Ababa [19], 38.9 and 1.2% in central high land of Ethiopia [20], 26.36 and 3.95% in North Gondar [21], 42.1 and 9% in Eastern part of Amhara region [22], 44.6 and 3.9% in Bahirdar [23], 38.9 and 1.2% in central highland of Ethiopia [24] and 47.24 and 6.29% in Kallu province [25] were the prevalence of subclinical and clinical mastitis respectively. In the current study prevalence of mastitis both subclinical and clinical cases were remarkably high.

Variation with most of clinical cases and some agreements were observed to subclinical mastitis. This variability could be attributed to interaction of factor of management and environment to factor of animals and causative agent. The high prevalence in this study was caused by poor milking procedure (improper use of udder disinfectant, pre milking strip cup and post milking teat dipping) and lack of hygienic milking order and absence of dry cow therapy.

The prevalence of bovine mastitis in the present study was lower than those reported in some previous studies [26, 27]. According to Tesfaye *et al.* [28]; the prevalence of subclinical and clinical mastitis was 67 and 22.5% in Debrezeit and 69.9 and 42% [28] respectively. In another study, Workinesh *et al*. [29] reported that clinical mastitis was the second most frequent disease next to reproductive diseases.

In this study the prevalence of subclinical mastitis at cow level was 43.3% and this result is in agreement with the report of Girum [22] in eastern part of Amhara, Gizat *et al*. [23] in Bahirdar and Tesfu et *al.* [24] in central high land of Ethiopia which was 42.1, 44.6 and 38.9%, respectively. But in a study carried out in Tanzania as high as 90.3% subclinical mastitis prevalence was reported [30]. On the other hand an increased level of prevalence, 45.5, 46.6%, 63 and 68.1% in commercial farms in Ethiopia, Peri urban area of Addis Ababa Cross, HF and Jersey cows in Ethiopia were reported by Sori *et al*. [31]; Mungube *et al*. [32], Kerro and Tarekegn [33] and Zerihun [34] respectively.

In the current study, parity of cows, lactation stage and location of the udder had a significan influence on the prevalence of mastitis. The risk of mastitis increased with increasing number of parity. This study agrees with the research findings by Matios *et al*. [35]; Mekibib *et al*. [4] and Gizat *et al*. [23]. According to Busato *et al.* [36], the risk of clinical and subclinical mastitis increases significantly with advancing age of the cow, this could be due to the immunosuppression occurring with the advancement in age followed by giving many calves. The significant effect of stage of lactation on prevalence of sub-clinical mastitis in this study was also reported by Nesru [18], Mungube *et al*. [32], Kerro and Tareke [33] and Biffa *et al*. [1] in Ethiopia.

The high prevalence in cow at their third and above lactation could be due to increasing ease of penetration of the teat duct by pathogens and accumulated previous infection [9]. It is postulated that younger animals are less susceptible; through a more effective host defense mechanism. Older cows especially after four calving are more prone to mastitis [37].

Out of 146 positive samples, 109 were cultured positive. Among this cultured positive samples, five genera of bacteria and mixed bacterial infection were isolated (Table 3). The most prevalent mastitis causing agents isolated in this study were (31.19%) *Staphylococcus aureus*, (26.6%) coagulase negative staphylococci, *(*8.26*%) Streptococcus, (*11.93*%) E. coli,* (2.75*%*) *Corynebacterium and* (5.5*%) Klebsiella. S. aureus* was the predominant isolate from clinical and subclinical mastitis cases. This coincides with the results found by Mekibib *et al*. [4] and Tesfaye *et al*. [28]. Lower level of prevalence of *S. aureus* was also reported by Tolossa [25], Zerihun, [34] and Ranjan *et al*. [7] which was 25.6, 23.1 and 27.37% respectively.

High prevalence of *S. aureus* was also reported by Mekibib *et al*. [4], Harini and Sumathi, [38] and Hunderra *et al*. [24]. The high prevalence of *S. aureus* most likely is attributed to the wide distribution of the organism inside mammary glands and on the skin of teats and udders [40]. *S. aureus* has adapted to survive in the udder and establish chronic and subclinical infections. From there it is shed into the milk, which serves as a source of infection for healthy cows during the milking process [9]. The high prevalence of this organism may be associated with the frequent colonization of teat, its ability to exit intracellulary and localize with in micro-abscesses in the udder and hence resistance to antibiotic treatment [41].

Streptococcal species identified in the present study (8.26%) was lower than reported by Zerihun [34], (27%) and Tolossa, [25] (43.5%). This lower prevalence in the current study might be partly associated with widespread use of penicillin in the area, which is known to be effective to eradicate mastitis caused by udder/teat injuries (85.7%) reported in *Streptococcus* species [31]. This is in agreement with Mekibib *et al*. [4] (7.2 %).

Risk of mastitis varies from breed to breed. High yielding cows are generally considered to be more susceptible to intramammary infection e.g. Holstein Frisian (HF), Jersey or HF and Jersey cross bred dairy cows are more susceptible to mastitis than (Zebu) breeds of cows, it might be due to more resistance to disease and they are low milk producer than cross bred cows. Increased risk of clinical mastitis in Friesian compared with Jersey and Ayrshire heifers [42, 43].

In this study the overall prevalence of subclinical and clinical mastitis was 43.3 and 22% at cow level and 26.8 and 15.1% at quarter level respectively. Among the cultured positive samples, the most prevalent mastitis causing agents isolated were *Staphylococcus aureus* and coagulase negative staphylococci. Mixed infections were *Staphylococcus and Streptococcus, Staphylococcus and Corynebacterium, Staphylococcus and E. coli* and *Corynebacterium* and *E. coli.* Risk factors analysis revealed that prevalence of mastitis was significantly associated with parity, lactation stage and location of the udder.

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